Research Note

Attachment of *Pasteuria penetrans* spores on populations of *Meloidogyne javanica* and *M. incognita* virulent and avirulent on the *Mi* gene of resistant tomato

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Summary

Spore attachment of three *Pasteuria penetrans* isolates was assessed on juveniles of *Meloidogyne incognita* and *Meloidogyne javanica* populations from Greece. The nematode populations differed in their ability to reproduce on tomatoes with the *Mi* gene, conferring resistance to root-knot nematodes. The numbers of attached spores did not discriminate virulent from avirulent populations within each species. The differences in attachment rates probably reflect the specificity of *Pasteuria penetrans* to different *Meloidogyne* populations of the same species, within a country or even the same area.

Key words: biocontrol; root-knot nematodes

Pasteuria penetrans (*Pp*) is a mycelial and endospore forming bacterium parasitic in phytonematodes (Sayre & Starr, 1985) with potential as a biocontrol agent of root-knot nematodes (RKN, *Meloidogyne* spp). Isolates of the parasite seem to be highly specific to different *Meloidogyne* species and even to different populations of the same species (Stirling, 1985). Furthermore, rates of attached spores of certain *Pp* isolates could differentiate avirulent *M. incognita* and virulent on resistant tomato *M. incognita* – like populations with the last receiving fewer or no spores (Fargette *et al.*, 1994). The aim of the work described here is to investigate whether RKN populations from Greece, virulent and avirulent on the *Mi* gene of resistant tomato, differ in their susceptibility to spore attachment by three *Pp* isolates.

The populations of *M. javanica* and *M. incognita* (Tzortzakakis *et al.*, 1999, 2006) are maintained in potted tomatoes in a controlled environment and juveniles (J_{2s}) are collected after incubating egg masses in extraction filters. The tested *Pp* isolates originated from South Africa (*Pp*3, V. Spaull), Malawi (*Pp* M, A. Daudi) and Ivory Coast (*Pp* IC, S. Gowen). These had been maintained in University of Reading, UK in the form of dried roots containing spore

filled females (Stirling & Wachtel, 1980) after multiplication on various *M. javanica* and/or *M. incognita* populations for several years. Spore suspensions had been prepared by suspending ground root material in water. For attachment tests, equal dilutions (no of spores per ml) were made from each *Pp* isolate and distributed in 3 cm Petri dishes in which nematode J_{2s} were added. The volume adjusted to be equal in each dish containing the same number of spores (c. 20,000) and juveniles (c. 100) and incubated for 24 h at 25 – 27 °C. Observations were made on the number of spores attached on ten juveniles in each of the six replicate dishes per treatment using an inverted microscope. Treatment effects were assessed by ANOVA and compared with LSD test.

Two experiments were conducted with *M. incognita* and three with *M. javanica* populations. The isolates of *Pasteuria* differed significantly in all experiments with the *Pp*3 attaining higher attachment levels in all cases.

Initially, spore attachment was compared on a virulent and a non-virulent *Meloidogyne* population from each species. That differentiated the virulent *M. incognita* (*Mi*C1) on which significantly more spores attached than the non virulent (*Mi*33) (Table 1, Experiment 1). However, when more populations of *M. incognita* were assessed, there was no clear differentiation; the virulent *Mi*C1 had similar attached spores with the non-virulent *Mi*C2 and *Mi*K1, while spore attachment on the non virulent *Mi*33 and *Mi*5 was higher from the other (Table 1, Experiment 2). A comparison of the only virulent with the avirulent (grouped) populations indicated that there was no significant difference in attached spores (Table 1, Experiment 2). An unclear pattern was also recorded for the *M. javanica*. While spores attached in equal rates to both virulent (*Mj*C1) and non-virulent (*Mj*6/3) populations (Table 2, Experiment 1), the non-virulent *Mj*3 had a quite significant higher spore number ($P < 0.001$) from the three virulent *Mj*P1, *Mj*P2 and *Mj*4 (Table 2, Experiment 2). When six

Table 1. Spore attachment of *P. penetrans* spores on populations of *M. incognita* from Greece with different virulence on resistant tomato with the *Mi* gene

Nematode	Origin	Virulence on <i>Mi</i> gene	Experiment 1				Experiment 2			
			<i>Pp3</i>	<i>PpM</i>	<i>PpIC</i>	Mean	<i>Pp3</i>	<i>PpM</i>	<i>PpIC</i>	Mean
<i>Mi</i> 33	Crete	No	3.7	1.7	1.3	2.2	9.1	1.9	5	5.4
<i>Mi</i> C1	Crete	Yes	9.9	2.9	4.7	5.8	7	0.9	2.7	3.5
<i>Mi</i> C2	Crete	No	-	-	-	-	5.3	2.5	3	3.6
<i>Mi</i> K1	Peloponisos	No	-	-	-	-	7.9	1.2	2.2	3.8
<i>Mi</i> 5	Crete	No	-	-	-	-	8.5	1.9	3.9	4.8
Mean			6.8	2.3	3		7.6	1.7		3.4
LSD 5%				1.02				1.21		
LSD 1%				1.38				1.61		
P nematode effect				< 0.001				< 0.001		
P <i>Pp</i> effect				< 0.001				< 0.001		
P interaction				< 0.001				< 0.05		
P virulent–avirulent				< 0.001				> 0.05		

Each mean is the average of six replicates with 10I₂₅ each; LSD and P values from ANOVA

Table 2. Spore attachment of *P. penetrans* spores on populations of *M. javanica* from Greece with different virulence on resistant tomato with the *Mi* gene

Nematode	Origin	Virulent on <i>Mi</i> gene	Experiment 1				Experiment 2				Experiment 3			
			<i>Pp3</i>	<i>PpM</i>	<i>PpIC</i>	Mean	<i>Pp3</i>	<i>PpM</i>	<i>PpIC</i>	Mean	<i>Pp3</i>	<i>PpM</i>	<i>PpIC</i>	Mean
<i>Mj</i> 6/3	Crete	No	15.3	5.6	8.1	9.7	-	-	-	-	10.4	3	5.3	6.2
<i>Mj</i> C1	Crete	Yes	13.3	5.2	7.7	8.7	-	-	-	-	8.3	2	3.2	4.5
<i>Mj</i> P1	Epirus	Yes	-	-	-	-	4.7	1.5	1.6	2.6	4.7	1	1.6	2.4
<i>Mj</i> P2	Epirus	Yes	-	-	-	-	6.8	2.2	3.7	4.2	8.9	1.9	3.9	4.9
<i>Mj</i> 4	Crete	Yes	-	-	-	-	7.4	2.5	4.5	4.8	7.8	1.4	3.8	4.3
<i>Mj</i> 3	Crete	No	-	-	-	-	17.6	11.2	13.1	13.4	11.8	4.1	7.4	7.8
Mean			14.3	5.4	7.9		9.1	4.4	5.7		8.7	2.3		4.1
LSD 5%				3.47				1.78				2.26		
LSD 1%				4.68				2.37				3		
P nematode effect				> 0.05				< 0.001				< 0.001		
P <i>Pp</i> effect				< 0.001				< 0.001				< 0.001		
P interaction				> 0.05				> 0.05				> 0.05		
P virulent –avirulent				> 0.05				< 0.001				< 0.001		

Each mean is the average of six replicates with 10I₂₅ each; LSD and P values from ANOVA

populations of *M. javanica* were tested, spore attachment on non-virulent *Mj6/3* did not differ from that on virulent *MjC1*, *Mj4* and *MjP2* while the non virulent *Mj3* had significantly more attached spores than the three virulent (Table 2, Experiment 3). A comparison of the grouped virulent and avirulent populations in Experiments 2 and 3 indicated that significant lower spores attach on virulent (Table 2, Experiments 2 and 3).

The results suggest that there is no evidence of a relationship between spore attachment to juveniles and virulence to the *Mi* gene in populations of *M. javanica* and *M. incognita* from Greece. The observed differences between two populations become variable and inconsistent when more *Meloidogyne* populations are tested. That probably reflects the specificity of certain *Pp* isolates to different *Meloidogyne* populations of the same species within a country or even the same area (Stirling, 1985; Espanol *et al.*, 1997).

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